Computational Modeling to Evaluate Candidate Modes of Action for the Carcinogenicity of Arsenic

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1. Short Description of the Project

Inorganic arsenic (iAs) is a multi-site human carcinogen. The current cancer risk assessment for iAs is based on the default linear low dose extrapolation method using epidemiological data from Taiwan (USEPA. 2001). Default-based risk assessments are considered to be health protective but provide no assurance of accuracy, i.e., the predicted risk may be much larger than the actual risk. Overprediction of risk is important when remediation is expensive, as is the case with arsenic. Compliance with the current drinking water standard of 10 ppb has been estimated to cost from $195 to $675 million per year in the U.S.

The goal of this project is to develop accurate predictions of cancer risk associated with arsenic exposure while, of course, continuing to adequately protect the public health. Following the Guidelines for Carcinogen Risk Assessment (US EPA, 2005), a biologically based dose-response (BBDR) model for the human carcinogenicity of iAs will be developed. The model will maximize the use of mechanistic information on iAs pharmacokinetics and pharmacodynamics, thereby increasing the accuracy of predictions of dose-response behaviors. The BBDR model will be able to predict the shape of the dose-time response surface for any exposure scenario of interest.

2. What is the EPA Context for the Project?

EPA, through its Office of Water (OW) and National Center for Environmental Assessment (NCEA), regulates allowable concentrations of iAs in drinking water. The EPA's National Health and Environmental Effects Research Laboratory (NHEERL) conducts a sizeable laboratory research program - 19 P.I.'s with 15-20 FTE's, including support staff - on the mechanisms of arsenic carcinogenicity. The NHEERL P.I.'s are collectively an important resource for expertise on mechanisms of iAs carcinogenicity. The laboratory research effort has not always, however, been as focused as it could have been on relevance to regulatory issues. The BBDR Project is intended to address this shortcoming. Starting in early 2006, a still-ongoing series of joint meetings of NHEERL and NCCT staff have been held at which the state of arsenic science was reviewed and principles for the design of experiments that efficiently support BBDR modeling were developed. These design principles are now in use in NHEERL. NHEERL Senior Management is also actively involved in the process, and have committed to ensuring that resources will be made available to support the newly planned research. These developments will serve to coordinate laboratory research in NHEERL with BBDR model development in the NCCT and with the regulatory needs of OW and NCEA. Under the 2005 Cancer Guidelines, a biologically based model is the preferred tool for cancer risk assessment. This project will thereby maximize the relevance of NHEERL research to the regulatory activities of OW and NCEA.

The Safe Drinking Water Act, as amended in 1996, requires that the Agency review the existing National Primary Drinking Water Regulation for iAs no less often than every six years. A usable version of the BBDR model is needed by 2012 for use in the Six-Year Review to be completed in 2015. (The current Six-Year Review, to be completed in 2009, does not allow enough time for new data collection and model development.)

3. What are the Strategic Directions and Science Challenges?

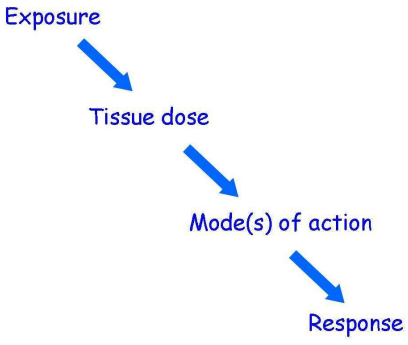

Figure 1. Main elements of a BBDR model. A PBPK model describes the relationship between exposure and tissue dose. The mode of action links the tissue dose that is predicted by the PBPK model with the response of interest.

A BBDR model combines a physiologically based pharmacokinetic (PBPK) model with a description of one or more modes of action that link tissue dosimetry, as predicted by the PBPK model, with endpoints of regulatory interest (Fig 1). BBDR modeling is motivated by the fact that, for a given chemical such as iAs, the relationship between exposure and response is determined by biological processes. These processes are conveniently categorized as either pharmacokinetic (PK) or pharmacodynamic (PD). Development of the BBDR model requires quantitative characterization of the biological mechanisms of PK and PD at a level of detail consistent with available data.

While exposure to iAs is associated with cancer and noncancer health effects, the primary focus of this project is on cancer. The endpoints of primary concern are bladder, lung, and skin cancer.

Development of a BBDR model describing several cancer endpoints might seem to pose an insurmountably large challenge due to the potential complexity of the model. We think, however, that this is not the case since some model components will be shared. The PBPK model is not endpoint-specific, and will constitute a shared front end for all cancer endpoints. It is also likely that one or more key events (e.g. oxidative stress), may be shared between different endpoints. The BBDR models for different cancer endpoints will, therefore (1) share the PBPK model front end, (2) probably share, at least partially, mode of action descriptions, and (3) diverge at the level of tissue-specific effects (Fig. 2).

Available data and data needs

Literature review and expert consultation are being used to identify the best supported modes of action. Well-established key events in the modes of action for iAs include oxidative stress, cytotoxicity, altered DNA repair, altered growth factors, altered cell proliferation, chromosome damage, and altered DNA methylation (Fig. 3). Available data from laboratory animals and from human epidemiology that can be directly used for development of the BBDR model or have potential supportive value for model calibration were reviewed. The strengths and weakness of these data are summarized in the following:

Strengths of the available database with respect to BBDR modeling

- Relatively good human tumor datasets exist for iAs, though the accompanying exposure data may not be as robust as we would like (Berg, Burbank, 1972; Cebrian, Albores *et al.*, 1983; Chen, Kuo *et al.*, 1988; Wu, Kuo *et al.*, 1989; Engel Smith,
- 1994; Bates, Smith *et al.*, 1995; Chiou, Hsueh *et al.*, 1995; Tollestrup, Daling *et al.*, 1995; Tsuda, Babazono *et al.*, 1995; Guo, Lipsitz *et al.*, 1998; Kurttio, Pukkala *et al.*,

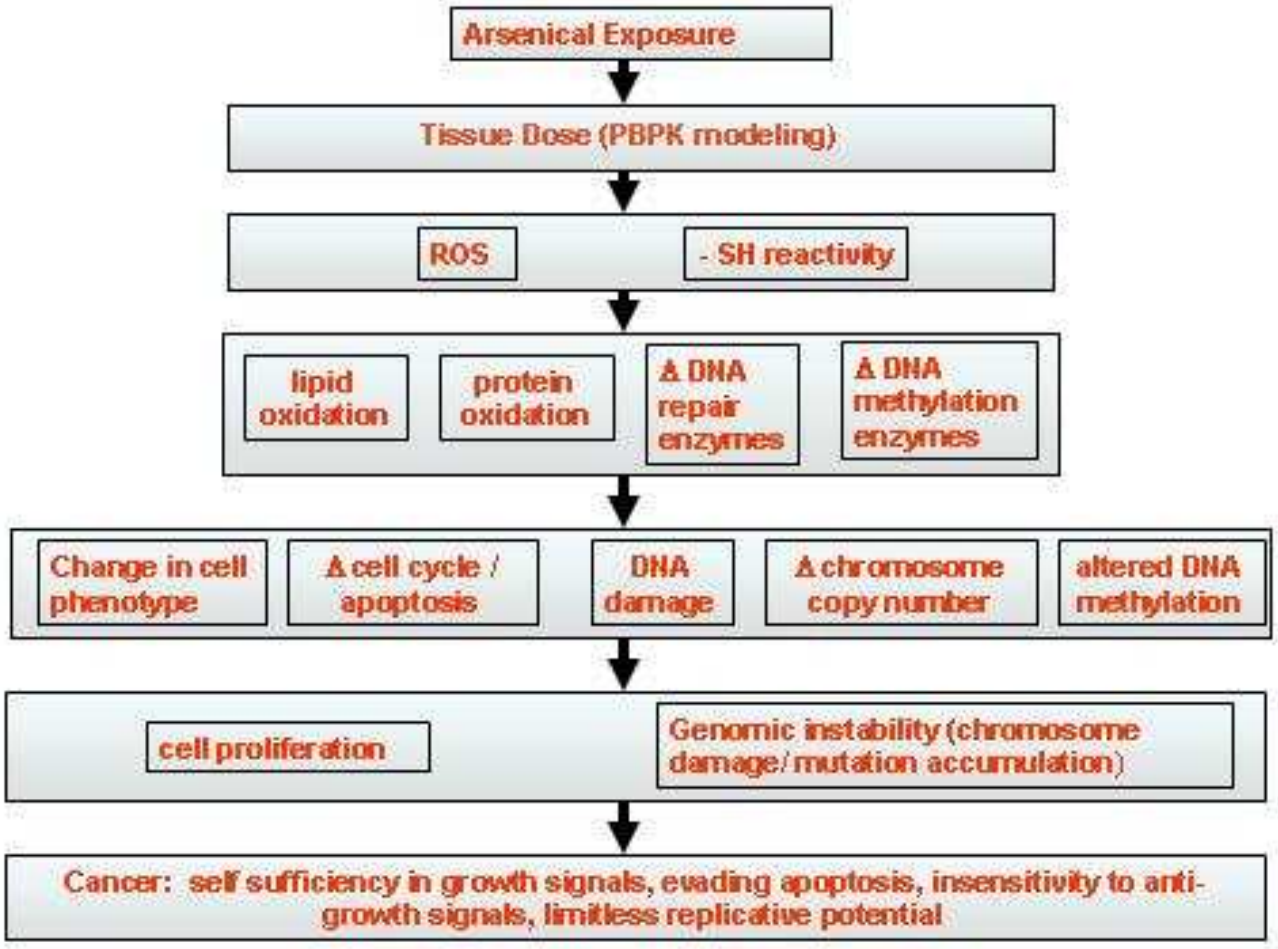

Figure 3. Multiple modes of action and possible interrelationships. A variety of modes of action have been implicated in the adverse health effects of iAs. It is not clear at this time, however, how the individual modes should be ranked with respect to dose and time course. It seems likely that one or at most a limited number of modes occurs at lower doses and earlier time points than the other modes. Ranking of modes with respect to activating doses and time courses will be an important component of future research designed to support development of the BBDR model.

- 1999; Lewis, Southwick *et al.*, 1999; Ferreccio, Gonzalez *et al.*, 2000; Chiou, Chiou *et al.*, 2001; Moore, Lu *et al.*, 2002; Guo, Yu *et al.*, 2001; Karagas, Stukel *et al.*, 2001; Karagas, Tosteson *et al.*, 2004; Lamm, Engel *et al.*, 2004; Michaud, Wright *et al.*, 2004.).
- The human noncancer database is developing rapidly and is likely to be of sufficient quality for both exposure and response endpoints.
- PBPK models have been developed for both rodents and humans (Clewell, Thomas *et al.*, 2007).
- A variety of potential key events have been implicated in the biological effects of iAs (though these data are of limited utility for BBDR modeling– see below) (Fig. 3). These key events can currently be organized into a variety of modes of action (Beckman, Beckman *et al.*, 1977; Petres, Baron *et al.*, 1977; Nordenson, Beckman *et al.*, 1978; Li, Rossman, 1989;.Lee-Chen, Gurr *et al.*, 1993; Warner, Moore *et al.*, 1994; Yamamoto, Konishi *et al.*, 1995; Kitchin, 1996; Brown, Yager, Wiencke, 1997; Germolec, Spalding *et al.*, 1997; Gonsebatt, Vega *et al.*, 1997; Zhao, Young *et al.*, 1997; Hu, Su *et al.*, 1998; Wanibuchi, 2000; Yamanaka, Mizol *et al.*, 2001.).

Weaknesses of the available database with respect to BBDR modeling

For a given exposure scenario, BBDR models can predict the associated curves for exposure-response and time course of response. These capabilities thus allow time-response surfaces to be generated. Accordingly, development of robust BBDR models requires adequate dose-response and time course data for key model parameters. Although the literature on adverse health effects of iAs in humans and laboratory animals is extensive, it tends to be weak with respect to these very characteristics that are critical to model development – exposure-response and time course characterizations. Key issues with respect to the currently available mode of action data are:

- Lack of data that supports ranking of candidate modes of action by dose and time course – the key event that occurs at the lowest dose and earliest time point relative to other key events is most likely to be a mechanistic driver for cancer and noncancer effects. Higher dose and later time point events are more likely to be secondary to the primary mode of action (Fig 3).
- Limited dose-response and time-course data for key events in any given mode of action.
- Little guidance on how data generated in vitro should be adjusted for use in a model that describes in vivo processes.

Research needs and experimental designs

EPA-NHEERL staff has considerable expertise in design and conduct of laboratory studies on the mechanisms of iAs-induced toxicity and carcinogenicity. We have developed a research planning process that uses this expertise for design of future laboratory that will be conducted in support of BBDR modeling. The ORD iAs BBDR modeling project has provided guidance to NHEERL staff on the characteristics of experimental designs that optimally support BBDR modeling. If the weaknesses in the current database identified above can be addressed by new research we will have the opportunity to develop a robust BBDR model and to significantly reduce uncertainties in iAs risk assessments for cancer. To this end the following recommendations have been developed:

1. Identify the key events that describe a mode (or modes) of action that operate at the lowest dose levels and the earliest time points. Characterization of events that operate at higher dose levels and later time points will still be useful, as these events may link the initial effects of iAs with its carcinogenic effects.
2. A mode of action should be characterized by one or more key events. If more than one key event defines a mode of action, then the sequence of the events should be specified for that mode of action (Fig. 4).
3. Key events should be characterized by dose-time-response surfaces.
4. Data are preferably generated in vivo. If data are generated in vitro, then specific guidance should be developed on quantitative extrapolation to the in vivo situation.
5. Dose response studies should include use of target tissue doses that are close to those thought to occur in people who suffer adverse health effects from iAs exposure. PBPK modeling can be used to help identify these concentrations.

Coordination of experimental designs between groups studying different modes of action is essential.

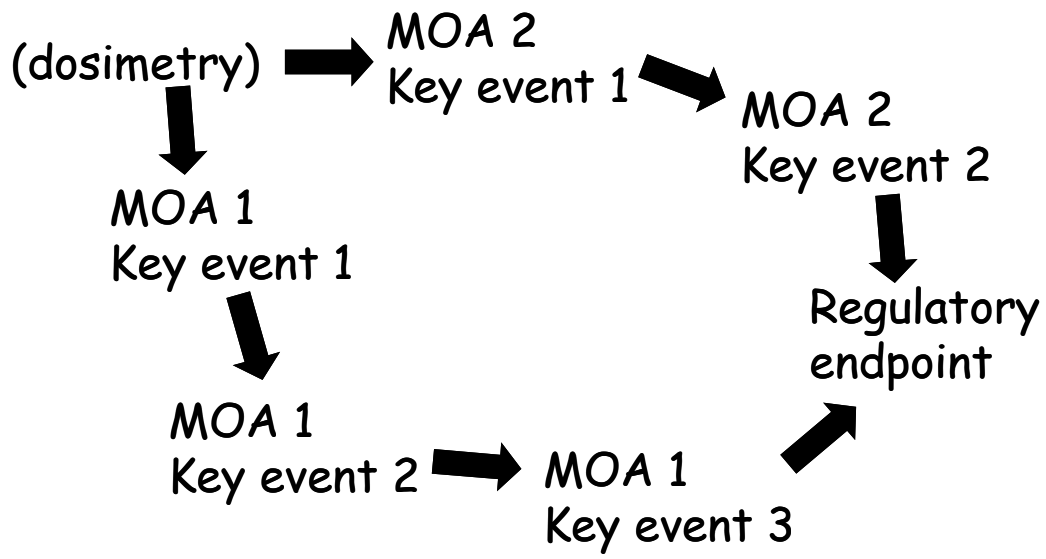

Figure 4. Conceptual scheme where 2 modes of action (MOA1, MOA2) link iAs dosimetry with a regulatory endpoint. Rigorous characterization of the dose- and time-response relationship between dosimetry and the regulatory endpoint requires identification of the key events, their sequences, and characterization of the dose- and time-responses for the key events. Although not indicated in this figure, interactions between the 2 modes of action are also possible.

Significance of the BBDR Model for Risk Assessment

Developed appropriately, the BBDR model will be the preferred tool for predicting cancer risk at environmentally relevant levels of exposure. It is important to recognize, however, that risk predictions provided by such a model may not differ from those obtained with other, less biologically motivated models. Rather, the return on investment obtained with a BBDR model will be greater confidence in the accuracy of its risk predictions. Accuracy is important because the costs of compliance with regulatory standards for iAs are high. The more accurate the prediction of risk, the greater is our confidence that predicted risk and the associated costs of compliance are commensurate with the actual risk. BBDR modeling provides this confidence because it makes maximal use of information on the biological processes that determine the shape of the dose-response curve. BBDR modeling is different in this regard from alternative approaches that rely to a greater extent on default assumptions about the shapes of carcinogen dose-response curves. The alternative approaches are generally though to be protective of the public health but provide no confidence that risk predictions are accurate, and thus no assurance that the costs of compliance are aligned with the actual health risk.

4. What are the Short-Term (1-2 year) and Long-Term (3-5 year) Goals?

The main shorter-term (1-2 year) goal is to establish the coordinated program of laboratory research in support of development of the BBDR model. Development of the BBDR model will not be possible without the new data. We do plan, however, to begin preliminary development of the BBDR model without waiting for the new data. This initial effort will include multistage clonal growth modeling of human cancer mortality and incidence data to identify how exposure to iAs affects the parameters of the clonal growth model. This will then allow us to examine how target tissue dosimetry of iAs and its methylated metabolites, as predicted by

PBPK modeling, is correlated with the cell division and mutation parameters of the clonal growth model. As mode of action data become available from ongoing laboratory research, it will be incorporated into the BBDR model to refine the linkages between target tissue dosimetry and the relevant parameters of the clonal growth model.

The main longer-term (3-5 year) goal is to develop a version of the BBDR model that is sufficiently robust to be acceptable for use in risk assessment. Development of the model over the coming 3-5 years is, however, likely to identify new questions and issues that will only be addressable by additional laboratory research and continued development of the BBDR model. At that point in time Senior Management will need to decide if the potential return from continued work justifies the additional cost.

We anticipate that, over the next 5 years, the project will provide a substantial amount of new information about the carcinogenic mode or modes of action of iAs and, as well, new understanding of technical issues in development of BBDR models. It will thus be important to actively communicate the significant achievements of the project in an ongoing basis at scientific meetings and in the peer-reviewed literature.

5. What other Components of EPA or Outside Organizations are Involved?

NHEERL
- Steve Edwards
- Doug Wolf

Environmental Carcinogenesis Division of NHEERL
- James Allen
- Don Delker
- Yue Ge
- Kirk Kitchin
- Andrew Kligerman
- Gail Nelson
- Stephen Nesnow
- Pedro Ortiz
- Barbara Roop
- Sheau-Fung Thai
- Witold Winnik

Experimental Toxicology Division of NHEERL
- Hisham El-Masri
- Michael F Hughes
- Elaina Kenyon
- David Thomas

Human Studies Division of NHEERL
- Rebecca Calderon
- Judy Mumford

Representatives from the National Center for Environmental Assessment, the Office of Water, and the Office of Prevention, Pesticides and Toxic Substances have attended planning meetings and have provided input from their perspectives on desirable characteristics of the BBDR model.

6. How is Data Management Being Achieved?

The segment of this project that is within NCCT is limited to computer programming – the actual development of the BBDR model. The main data management concerns are archiving and backup of computer programs, and organization and storage of the data used to support model development.

Computer programs are archived to a "backup" directory on a regular basis whenever significant changes are made to the code. Backups are placed in a folder named with the date of the backup and the files are converted to "read-only" status. The hard drive on which the working files and the backup files are located is itself backed up on a daily basis to an external drive.

A Microsoft Excel spreadsheet is used to store the data used for model development. This spreadsheet is backed up in the same manner as the programs comprising the BBDR model.

7. What are Appropriate Measures of Success?

The main long-range measure of success for this project will be use of BBDR model-generated health risk predictions in risk assessment for iAs. Successful development of the BBDR model for iAs, but without acceptance of the BBDR model by regulators, would still be a significant technical achievement, but would nevertheless be a failure in terms of immediate impact on public health.

Development of the BBDR model will depend on the acquisition of new data. An important measure of success will thus be the organization and completion of this targeted data collection effort. This measure of success has a significance that extends beyond the iAs project, as it will demonstrate our ability to coordinate laboratory research within NHEERL with computational modeling in support of risk assessment. In this way the current project will serve as a prototype for similar efforts with other compounds.

Another measure of success will, of course, be description of the BBDR model in the peer-reviewed literature. This latter measure of success will be important for ensuring that the technical aspects of model development and new insights gained about the carcinogenic mode or modes of action of iAs are transmitted to the larger scientific community.

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